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Endocrine-Related Cancer

Commentary

Leptin and Cancer: From Cancer Stem Cells to Metastasis

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14. ABSTRACT

There is growing evidence that obesity is a risk factor of cancer incidence and mortality. Hence,the identification of the mechanistic links between obesity and cancer progression is emerging as a topic of widespread interest. Recently several groups have addressed the functional roles of leptin, an adipocyte-derived adipokine, for mammary tumor progression. In this issue of Endocrine-Related Cancer,Zheng et al. study the role of leptin on tumor growth in a xenograft model of MMTV-Wnt1 derived cancer cells. They study growth of these cancer cells in the context of obese animals, such as ob/ob mice (lacking leptin) and db/db mice (lacking functional leptin receptors) and find that leptin triggers leptin receptor positive cancer stem cell differentiation, thereby promoting tumor cell survival. These findings highlight the therapeutic potential for leptin and leptin signaling in the context of mammary tumor growth.

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Abstract

There is growing evidence that obesity is a risk factor of cancer incidence and mortality. Hence, the identification of the mechanistic links between obesity and cancer progression is emerging as a topic of widespread interest. Recently several groups have addressed the functional roles of leptin, an adipocyte-derived adipokine, for mammary tumor progression. In this issue of ***Endocrine-Related Cancer***, Zheng et al. study the role of leptin on tumor growth in a xenograft model of MMTV-Wnt1 derived cancer cells. They study growth of these cancer cells in the context of obese animals, such as ***ob/ob*** mice (lacking leptin) and ***db/db*** mice (lacking functional leptin receptors) and find that leptin triggers leptin receptor positive cancer stem cell differentiation, thereby promoting tumor cell survival. These findings highlight the therapeutic potential for leptin and leptin signaling in the context of mammary tumor growth.

A large number of epidemiological studies on cancer incidence link the propensity to develop certain types of cancers (such as colon, thyroid, esophagus, renal, endometrial, and postmenopausal breast cancer) with an individual's excess body fat/obesity (Bianchini, et al. 2002; Calle, et al. 2003). Adipokines, i.e. adipocyte-derived secretory proteins, represent likely candidates to mediate at least in part the increased cancer risk and enhanced progression associated with obesity. Other contributors to obesity-related cancer progression are the insulin/IGF-1 pathways and sex hormones (summarized in (Park, et al. 2011a)). Amongst the adipokines, leptin is the most intensively studied factor, both in metabolism in general as well as in obesity-related cancers due to the fact that leptin levels increase in proportion to fat mass. In this issue of ***Endocrine-Related Cancer***, Zheng et al. show that tumor cells derived from a widely used mammary tumor model, the MMTV-Wnt1 mouse, grow less effectively in leptin deficient, obese mice (generally referred to as "***ob/ob mice"***) compared to obese mice with an intact leptin pathway. This suggests that leptin signaling plays an essential role in MMTV-Wnt1 tumor cell growth and survival. They conclude that these leptin effects are mediated through a comprehensive set of responses of leptin receptor positive tumor cells that include a cancer stem cell population defined by characteristic cell surface markers that expresses the leptin receptor as well (Zheng, et al. 2011).

Leptin - Just in Your Head?

Leptin is encoded by the ***ob*** gene and is a well-established adipokine influencing appetite control and energy expenditure through its actions on the hypothalamus and other regions in the brain where leptin receptors are highly expressed (Frederich, et al. 1995; Halaas, et al. 1995; Vaisse, et al. 1996). A single transcript encoded by the ***db*** gene produces at least five different variants of the leptin receptor protein through alternative splicing (Lee, et al. 1996). However, only the long form of leptin receptor (LEPR-B) has a cytoplasmic domain that transduces the leptin-mediated downstream signaling events, such as activation of the PI3K, ERK1/2, and Jak2/Stat3 pathways (Baumann, et al. 1996; Morris and Rui

2009). In addition to the neuronal actions, leptin also exerts other physiological responses in peripheral tissues. These include effects on the immune response, angiogenesis, reproduction as well as an intracellular crosstalk with signaling pathways of growth hormones, such as insulin, and lipid metabolism pathways (Margetic, et al. 2002; Muoio and Lynis Dohm 2002; Sierra-Honigmann, et al. 1998). Adipose tissue with its well-appreciated endocrine functions takes advantage of leptin as a potent signaling molecule that profoundly impacts multiple peripheral tissues. Tumor tissues have been demonstrated to have cells that are leptin responsive, including tumor cells themselves. A number of reports indicate that leptin receptors are highly abundant in many tumor tissues compared to benign or normal tissues. Leptin-responsive tumors include mammary carcinoma, pancreatic- and gastrointestinal tumors, such as esophageal, gastric and colon cancer cells (Garofalo, et al. 2006; Howard, et al. 2010; Ishikawa, et al. 2004).

Leptin and the Breast Cancer Axis

Leptin is a growth hormone that plays an important role in development, differentiation and cell growth under normal physiological conditions. It affects a number of cell types, including neuronal cells, immune cells, pancreatic β cells, endothelial cells as well as adipocytes themselves. Leptin exerts its effects through LEPR-B mediated downstream pathways, such as PI3K, ERK1/2, and Jak2/Stat3 (Morris and Rui 2009). With respect to breast cancer, leptin is an attractive target due to its involvement in cell proliferation, migration and invasion, giving rise to more aggressive and metastatically more potent tumor cells (Cirillo, et al. 2008). ***In vitro*** studies using human breast cancer cell lines indicate differential leptin responses amongst various cell lines may depend primarily on receptor levels of LEPR-B amongst those cell lines studied.

Numerous attempts have been made to evaluate leptin effects on breast cancer progression ***in vivo***. Genetic loss of function mutants for leptin or the leptin receptor (i.e. ***ob/ob*** or ***db/db*** mice) develop systemic metabolic abnormalities that include obesity, diabetes, infertility as well as immune defects

(Friedman 2009). Manipulation of leptin levels with these genetic mouse models in MMTV-TGFα mice failed to develop mammary tumors, because these mice completely lack a ductal mammary epithelium. Since most tumors arise from the ductal epithelium, it is not possible to use these mice for the study of mammary tumor development (Cleary, et al. 2004; Cleary, et al. 2003). Recently, more compelling ***in vivo*** evidence was provided with a hypothalamic LEPR-B reconstitution in ***db/db*** mice (***db/db***$^{Nse+/+}$), which restores metabolic abnormalities in ***db/db*** mice, such as obesity, diabetes, and infertility. These mice also develop a normal mammary epithelium (Chua, et al. 2004). They can therefore be crossed with a mammary tumor model, such as the MMTV-PyMT mouse. Results from these crosses suggest that a LEPR-B mediated signal promotes tumor growth and metastasis. Cancer cell metabolism is affected in these mice by orchestrating downstream pathways, such as PI3K, ERK1/2, and Jak2/STAT3 (Park, et al. 2011b). Additionally, diet-induced obese mouse models have been used to modulate leptin levels ***in vivo***. From these diet studies, it is apparent that mammary tumors grow faster under high fat diet conditions. Consistent with a possible involvement of leptin, obese MMTV-TGFα mice do indeed display elevated circulating leptin levels (Dogan, et al. 2007). However, for obvious reasons, it is challenging to discern distinct leptin effects from other metabolic changes associated with obesity-induce metabolic dysregulation

Xenografts of MMTV-Wnt1 breast cancer cells transplanted into diet-induced obese mice (which maintain high levels of circulating leptin over prolonged periods of time) grow faster, in further support of a tight association between obesity and mammary tumor growth (Nunez, et al. 2008). To assess whether this obesity-associated increase is solely due to leptin or a combined effect of other metabolic parameters that change under these conditions, Zheng et al. in this issue of ***ERC*** demonstrate that xenografts of MMTV-Wnt1 cancer cells transplanted into leptin deficient obese mice (***ob/ob)*** displayed a stunted tumor growth. In contrast, transplants into obese ***db/db*** mice (lacking the leptin receptor and as a result displaying high leptin levels) augmented tumor growth. This is a clear indication that tumor cell behavior relies heavily on leptin signaling, even if cancer cells are exposed to other mitogenic signals and

nutrients excess, such as hyperinsulinemic, hyperglycemic and hyperlipidemic conditions prevailing in obesity (Zheng et al. 2011).

LEBR-B$^+$ Cell Populations in Mammary Tumor Tissues

Cancer progression is a multistep process that involves tumor initiation, primary tumor growth, invasion, and metastasis, with minimally the latter three relying on interactions with stromal tumor components that include endothelial cells, immune cells, fibroblasts and adipocytes (Hanahan and Weinberg 2011). The mechanistic details underlying the association between obesity and cancer as they relate to leptin, are still elusive despite the vast literature on the topic. A key question remains as to whether leptin contributes to cancer initiation or whether its role is restricted to promoting the growth of existing tumor cells? Numerous ***in vitro*** studies with breast cancer cell lines indicate that leptin directly contributes to LEPR-B positive cancer cell proliferation, migration and invasion. Furthermore, leptin has been known to regulate the immune response and angiogenesis through targeting immune cells and endothelial cells, respectively (La Cava and Matarese 2004; Sierra-Honigmann et al. 1998), an effect that clearly affects cancer cell growth as well, albeit only indirectly. Despite these potent leptin-induced cellular changes on mammary tumor progression at various stages, the specific leptin responsive cell populations in tumor tissues have not yet been adequately defined.

As xenografts of MMTV-Wnt1 cell into ***ob/ob*** mice failed to thrive, Zheng et al. analyzed these regressed tumor tissues and compared them to tumor cells isolated from wildtype mice to identify a leptin responsive cell population missing in the ***ob/ob*** population but present in the wildtype isolates (Zheng et al. 2011). Xenograft tumor tissues from transplants into ***ob/ob*** mice were analyzed by fluorescent activated cell sorting (FACS) for markers characteristic of cancer stem cell (CSC)-rich populations, such as CD29 (integrin $\beta1$), CD49f (integrin $\alpha6$) and CD24 (heat stable antigen) upon depletion of CD45 and Ter119 positive cells (Charafe-Jauffret, et al. 2009; Mani, et al. 2008). Interestingly, they found that the

survival of $CD29^{+}CD24^{-}$ CSC population is efficiently increased in response to leptin. They measured this by using a "tumorsphere formation" assay. A leptin responsive $CD29^{+}CD24^{-}$ CSC population expresses high levels of LEPR-B. These findings are highly provocative, but will require a more in-depth evaluation of this CSC population to strengthen the hypothesis that leptin is a mammary tumor-initiating factor on the basis of its ability to stimulate cancer stem cell survival.

Concluding Remarks

These results shed new light on the role of leptin and its receptor in mammary tumors (and potentially other LEPR-B^{+} tumor types). These observations touch upon the important question whether obesity can be "tumor initiating" and highlights that leptin may be an important contributing factor. Based on these results, an emerging model for the role of leptin on tumor progression is raised (**Figure 1**). Obesity via LEPR-B mediated signaling pathways promotes mammary tumor growth at various stages, affecting different tumor cell types that include a spectrum of cells from early cancer stem cells through metastatic tumor cells. In this model, leptin is involved at early stages in cancer stem cell survival. Once the primary tumor is established, leptin triggers cancer cell proliferation, migration and invasion. Furthermore, it exerts effects on tumor-associated stromal cells, such as endothelial cells, immune cells, and fibroblasts, to enhance angiogenesis and inflammatory processes that support tumor growth. Considering all of these potential roles for leptin on cancer progression, the leptin signaling pathway emerges as an attractive therapeutic target for the obese cancer patients. Can the peripheral leptin actions effectively be targeted without deteriorating the prevailing central leptin resistance under those conditions? In light of the life-threatening circumstances in the context of rapid growth of a tumor mass, a transient deterioration of central leptin action may be a price well worth paying.

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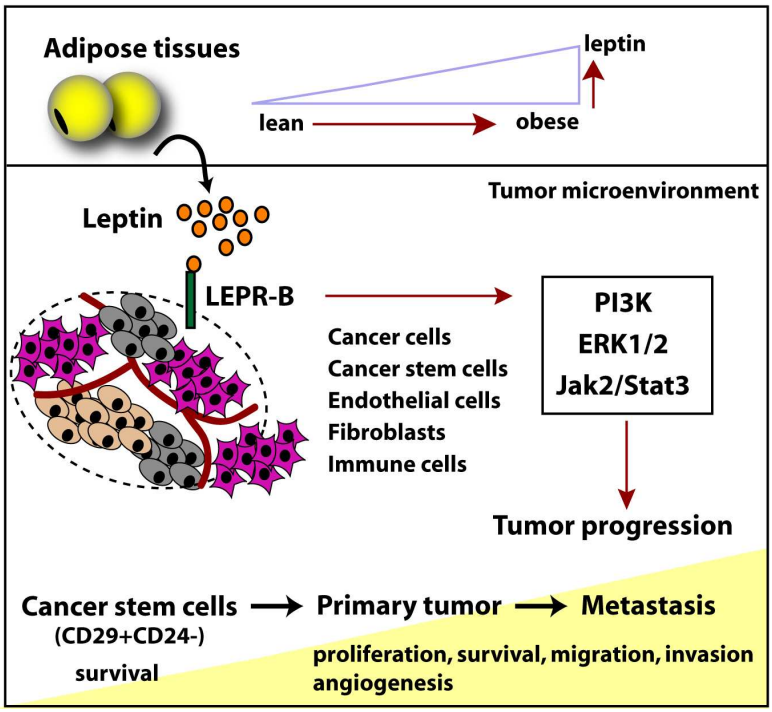
Figure legends

Figure 1. The potential role of leptin in mammary tumor progression. Leptin levels are increased in proportion to adipose tissue mass over the course of obesity. Leptin produced by adipose tissues binds to LEPR-B expressing cells within the tumor microenvironment, which include epithelial cancer cells, cancer stem cells, immune cells, endothelial cells and potentially fibroblasts. LEPR-B mediated pathways include activation of downstream kinases, such as PI3K, ERK1/2 and Jak2/Stat3. These pathways contribute to various steps of tumor progression, from cancer stem cell survival and proliferation to metastatic tumor growth.

Figure 1



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